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Short epitope-based synthetic peptides for serodiagnosis of human strongyloidiasis

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ABSTRACT

Strongyloidiasis is one of the major intestinal infections in humans, and a neglected tropical disease whose diagnosis still poses a challenge. We hypothesized that diagnostic tests based on short peptides containing major epitopes may represent a promising strategy to improve strongyloidiasis detection due to reduced cross-reactivity and higher sensitivity. Our aim was to evaluate two synthetic peptides selected by phage display (C10 and D3) as potential tools for serodiagnosis of strongyloidiasis, and to predict their putative antigen target. To investigate their diagnostic potential, we have tested different panels of serum samples (n = 120) by enzyme linked immunosorbent assay (ELISA) to detect specific IgG, and their diagnostic parameters were calculated. Similarities with proteins from *Strongyloides stercoralis* were searched and conformational epitopes were predicted and aligned to known protein structures. Both C10 and D3 achieved sensitivity of 95%, and specificities were 89.2% and 92.5%, respectively. D3 presented the highest diagnostic efficiency (93.3%). Epitope prediction for both C10 and D3 led to the alignment with the cytochrome c oxidase subunit 1 structure. In brief, we propose two synthetic peptides as new biomarkers for serodiagnosis of strongyloidiasis, which can be promptly used for ELISA and in future field sensor platforms.

1. Introduction

Global prevalence of *S. stercoralis* has been increasing in the past few years, especially in many known endemic areas of the disease [1]. Strongyloidiasis is one of the neglected tropical diseases listed by the World Health Organization [2], and is estimated to affect at least 370 million people worldwide [3]. The exact prevalence of strongyloidiasis is not known, but in many tropical and subtropical countries it may infect up to 60% of the population. Though endemic in some developing countries, strongyloidiasis still poses a threat to the developed world [1]. The lack of accurate diagnostic and monitoring tools is a significant problem for a more complete understanding of strongyloidiasis dynamics, risk factors and response to interventions [4].

Among the unique characteristics of this nematode are its ability to persist and replicate within a host for decades producing minimal or no symptoms, and its potential to cause life-threatening infections by dissemination and hyperinfection in debilitated and immune-compromised patients. Such immunosuppression is generally caused by the use of systemic corticosteroids to treat chronic obstructive pulmonary disease, asthma or other diseases, by HTLV-1 and HIV infection, and by organ transplantation, which may trigger a fulminant and lethal form of strongyloidiasis [5–7]. The uncontrolled dissemination of larvae has an associated mortality

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of 68.5% in cases of dissemination and 60% in hyperinfection, considering this high mortality rates there is an urgent need to improve diagnostic tests [8–10].

The immunodiagnosis, however, is affected by various factors, for example different antigens show variable sensitivity and/or specificity values, leading to the occurrence of false-positive results caused by cross-reactivity with other parasitic infections, such as filariasis, ascariasis, and acute schistosomiasis [1,11,12]. Serological assays are now widely available due to their increased sensitivity, and when compared with parasitological methods, they present great potential to be used in multiple helminthic infections [1]. However, the development of specific antigens is still necessary in order to avoid cross-reactions. Therefore, attempts to produce recombinant antigens and synthetic peptides with high sensitivity and specificity are of great interest in the serological diagnosis of strongyloidiasis [13].

We have obtained promising results with phage-displayed mimotopes [14], which sequences were used to synthesize two epitope-based synthetic peptides (C10 and D3). Herein, we demonstrated the usefulness of these peptides to detect specific IgG in serum samples from patients with strongyloidiasis and predicted the conformational epitope on *S. stercoralis* cytochrome c oxidase subunit 1 with combined input of C10 and D3 sequences.

2. Materials and methods

2.1. Ethics statement

This study was conducted according to the ethical guidelines of the Brazilian Health Ministry after being approved by the Research Ethics Committee of the Federal University of Uberlandia (UFU), State of Minas Gerais, Brazil. The study was approved by the Research Ethics Committee from UFU under the number 553/09. Serum samples were obtained at the Clinics' Hospital of UFU.

2.2. Study population

This study used a panel of 120 serum samples divided into three groups. Group 1 (G1) consisted of 40 patients living in an endemic area and with confirmed parasitological diagnosis of strongyloidiasis by using Baermann and Moraes method [15,16], based on positive larval thermo-hydrotropism and Lutz method [17], a gravity sedimentation technique. Group 2 (G2) included 40 patients with positive diagnosis of other parasitic diseases including *Ascaris lumbricoides* (n = 7), *Enterobius vermicularis* (n = 5), *Giardia lamblia* (n = 3), hookworm (n = 8), *Hymenolepis nana* (n = 7), *Schistosoma mansoni* (n = 3), *Taenia* sp. (n = 5) and *Trichuris trichiura* (n = 2). Samples from Groups 1 and 2 were obtained from patients affected by single infection. Group 3 (G3) contained samples from 40 apparently healthy individuals based on their clinical observation, without evidence of contact with *S. stercoralis* infection or previous history of strongyloidiasis and three fecal samples tested negative.

2.3. Peptide design and synthesis

The peptide design was based on mimotopes sequences (C10 and D3) originated from phage display selections, as previously described by our group [14]. Each synthetic peptide consisted of two repetitive epitope motifs spaced by GGGS, followed by modifications with BSA coupled to the N-terminal and an amide to the carboxy-terminal. Peptides were synthesized at Peptide 2.0 (peptide2.com). Molecular weight and purity were 2377.67 and 92.97% and 2141.48 and 95.01% for C10 and D3, respectively.

2.4. Enzyme linked immunosorbent assay (ELISA) to detect specific IgG

Preliminary experiments were carried out to determine the optimal conditions for ELISA, through titration of peptides, serum and antibody conjugate.

Briefly, polystyrene microplates (Nunc MaxiSorp) were coated with 1 μg/mL of each peptide (C10 and D3) in carbonate bicarbonate buffer (pH 9.6, overnight, 4 °C). Microplates were washed once with phosphate buffered saline (PBS). Next, 50 μL/well of serum samples (G1, G2 and G3) diluted 1:160 in PBS containing 0.05% polysorbate 20 (PBS-T) and bovine serum albumin (BSA) 5% were added and incubated (60 min, 37 °C). After incubation, plates were washed six times with PBS-T, enzyme conjugate (goat anti-human IgG-peroxidase, Fc specific Sigma, St. Louis, USA) was added 1:2000 in PBS-T 0.05% – BSA 5% and incubated for 60 min at 37 °C. Plates were washed six times with PBS-T and the assay was developed, after another washing procedure, by the addition of hydrogen peroxide and orthophenylenediamine (OPD) in 0.1 M citrate phosphate Na_2HPO_4 buffer pH 5.5 for 15 min. The reaction was interrupted with H_2SO_4 (2 N). Optical densities (OD) were determined at 492 nm in an ELISA reader (TP-Reader ThermoPlate). All samples were tested in duplicates.

2.5. Statistical analyses

Data were analyzed using GraphPad software package 5.0 (GraphPad Software Inc., San Diego, USA). Receiver operating characteristic curves (ROC) defined the best cut-off value and described diagnostic parameters [18]. Area under curve (AUC) [19], sensitivity (Se), specificity (Sp), diagnostic efficiency (DE) and likelihood ratios (LR+ e LR−) were obtained from ROC tables. ELISA reactivity index (RI) was obtained by the ratio between OD and cut-off. Values of RI greater than the optimum point of reaction for each peptide were considered positive (RI > 1). Probability (P) values < 0.05 were regarded as significant and 95% confidence intervals (CI 95%) were provided. ANOVA (F) with Bonferroni post-test was used to determine differences in peptide reactivity among groups.

2.6. Bioinformatic analyses

To verify if our peptides could elicit comparable antibody responses raised by the original bacteriophage-fused antigens from *Strongyloides*, we have searched the *S. stercoralis* database for similarities through linear alignment with BLAST server (blast.ncbi.nlm.nih.gov), as previously defined [14]. The aligned proteins (one access number and one isoform, if repeated) were then submitted for homology analyses through the Swiss-Model structure modeling server (swissmodel.expasy.org), and the conformational epitope, recognized by C10 and D3 sequences, were predicted by the EpiSearch server (The Universtity of Texas Medical Branch; curie.utmb.edu/episearch.html). The Global Model Quality Estimation (GMQE) above 0.7 for peptides was considered for further analysis. The EpiSearch analysis was performed for peptide sequences, individually and in combination as input sequences, and epitopes that reached the highest score values were selected. Episearch analyses were performed in default modes, adjusting only patch size to the 7-mer phage library.

3. Results

3.1. Performance of the new antigens in the serodiagnosis of human strongyloidiasis

Fig. 1 shows the levels of specific anti-*S. stercoralis* IgG expressed in RI for serum samples from Groups 1, 2 and 3. For the C10 peptide,